REMARKS

Status of the Claims

Claims 1-33 were examined and stand rejected by the Examiner. In response to the Examiner's objection, claim 9 has been withdrawn. Applicants have amended claim 17 to be dependent from claim 5. These amendments do not add new matter, are intended to clarify the claims, and are not intended to limit their scope. Applicants amend and withdraw claims as noted without prejudice, and reserve the right to re-file the original claims to the application. Applicants request the Examiner to consider the following remarks in light of the newly amended claims.

The Invention

To place the following remarks in their proper context, Applicants summarize their invention as to methods for distinguishing between DNA species from different individuals, in the same biological sample, ***based entirely on epigenetic differences***. These methods do not require detection of the primary sequence of the DNA. Through the use of epigenetic differences, Applicants invention allows homologous DNA species from different sources to be distinguished even when the DNA homologues have identical primary sequences.

One method of identifying epigenetic differences is by chemically modifying the DNA sequence of a sample in a manner that is ***dependent upon*** the ***epigenetic difference***, then detecting the modification. For example, methylation of bases, an epigenetic difference, can be detected by methylation-specific PCR (MSP). MSP differs from routine PCR detection of nucleic acids in that, methylation -specific PCR includes the step of treating the DNA sample with bisulfite prior to amplification. Bisulfite treatment converts unmethylated cytosyl residues to uracyl residues. Methylated cytosyl residues are unaffected by bisulfite treatment. PCR-primers that ***recognize methylated DNA*** that has ***not been treated*** with bisulfite, ***will not recognize***

the same methylated DNA ***after bisulfite treatment***. A second set of primers specific for the DNA modified by bisulfite treatment are required to identify the modified DNA. Therefore, the second set of primers identifies the methylation pattern of the nucleic acid, not its primary sequence. This is illustrated by the fact that two populations of methylated DNA molecules with the same primary sequence but differing methylation patterns will be recognized by different primer sets after bisulfite treatment.

Epigenetic differences exist between paternal and maternal chromosome sets, ***of an individual.*** Epigenetic differences also exist between chromosomes ***of different individuals***. Consequently, potentially every chromosome of an individual includes epigenetic markers that distinguish nucleic acid from the individual from that of another individual. The instant invention is the first to show the application of this finding to differentiating between DNA species of ***different individuals*** in a common biological sample. This application is possible following Applicants' surprising finding that differential methylation patterns of DNA survive in a foreign host, even outside the cell.

Claim Rejections under 35 U.S.C. §112

Claims 21-23 are rejected by the Examiner as allegedly indefinite under 112 ¶2. According to the Examiner, indefiniteness arises as claims 21-23 are dependent from claim 15, which requires measuring the concentration of fetal DNA in maternal plasma or serum. It is unclear to the Examiner how imprinting by methylation will be ascertained by fetal DNA concentration, as recited in claim 15.

Applicants have amended claim 17, from which claims 21-23 directly depend, to be dependent from claim 5. This amendment removes the limitation the Examiner found confusing from claims 21-23.

Claim Rejections under 35 U.S.C. §102

Claims 1, 3-5, 8, 14-20, 24, 27 and 33 are not anticipated by Lo-WO.

Claims 1, 3-5, 8, 14-20, 24, 27 and 33 stand rejected by the Examiner as allegedly anticipated under 35 U.S.C. §102(b) by Lo *et al.*, (WO 98/39474) or Lo *et al.*, (U.S. Pat. No.: 6,258,540). The Examiner notes that the disclosure in both cited references is identical (referred to cumulatively hereinafter as "Lo-WO").

For a rejection of claims under §102 to be properly founded, the Examiner must establish that a single prior art reference either expressly or inherently discloses each and every element of the claimed invention. *See, e.g. Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986), *cert denied*, 480 U.S. 947 (1987); and *Verdegaal Bros. V. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). In *Scripps Clinic & Research Found. V. Genetech, Inc.*, 18 USPQ2d 1001 (Fed. Cir. 1991), the Federal Circuit held that:

> "Invalidity for anticipation requires that **all of the elements and limitations** of the claim are found **within a single prior art reference**.... There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Id.* at 1010.

Anticipation cannot be found, therefore, unless a cited reference discloses all of the elements, features or limitations of the presently claimed invention. Applicants respectfully submit that Lo-WO fails to recite all of the elements of claims 1, 3-5, 8, 14-20, 24, 27 and 33.

Claim 1 of the instant application, from which all other claims of the application depend, is to a method for differentiating between DNA species from different individuals in a biological sample using epigenetic differences between the DNA species. As noted in the Office Action, "epigenetic differences" is defined in the

instant application as being "any molecular or structural difference [in the nucleic acid] other than the primary nucleic acid sequence."

Lo-WO does not describe detection of epigenetic differences.

The Office Action summarizes Lo-WO as teaching a method of non-invasive prenatal diagnosis including sex determination and detection of pre-eclampsia by determining epigenetic differences between the DNA species. Applicants respectfully disagree and rebut the rejection.

Contrary to the Office Action summary, Lo-WO does not discuss methods for differentiating between maternal and fetal DNA based on epigenetic differences. Throughout the Lo-WO reference, the authors make clear that their methods detect paternally-inherited ***DNA polymorphisms or mutations*** not present in the maternal DNA (See, e.g., col. 5, lines 7-18 and the summary of the invention generally). DNA polymorphisms and mutations are differences in the primary sequence of DNA, not epigenetic differences. As the Examiner has noted, Applicants definition of "epigenetic differences" expressly excludes differences in the primary nucleic acid sequence of the molecules under study.

Specifically, the Office Action describes Lo-WO as determining the sex of a fetus by detecting the presence of a Y chromosome (col.2, lines 49-51), using PCR with Y-specific primers. Y-specific primers detect complimentary ***primary sequences*** unique to the Y-chromosome, ***not epigenetic differences***. Further, Applicants respectfully remind the Examiner there are no epigenetic differences to detect between fetal Y chromosome markers and a maternal homolog, as there is ***no maternal homolog to the Y chromosome.*** Lo-WO therefore cannot be differentiating fetal Y chromosome markers from maternal DNA through epigenetic marker differences.

The Office Action also describes Lo-WO as identifying a variety of diseases by detecting paternally-inherited DNA sequences that are not possessed by the mother (col. 2, line 57 to col 3, line 24), and other diseases identified by quantifying fetal

DNA in maternal blood fractions (e.g., col. 3, lines 25-57). In each case, ***fetal DNA is detected by*** screening for paternally-inherited ***primary sequence-based DNA markers*** using ***standard PCR*** techniques known in the art not designed to detect epigenetic differences. Applicants also respectfully remind the Examiner that DNA concentration is not "[a] molecular or structural difference other than the primary nucleic acid sequence," and therefore not an ***epigenetic*** difference.

As the sequence differences detected using the techniques of Lo-WO fall outside the definition of "epigenetic," Lo-WO fails to teach all of the elements of Applicants invention and the rejection should be withdrawn.

Claims 1, 3-4, 6, 27 and 33 are not anticipated by Lo-Lancet

Claims 1, 3-4, 6, 27 and 33 stand rejected by the Examiner as allegedly anticipated under 35 U.S.C. §102(b) by Lo *et al.*, Lancet, **351**, p. 1329-1330 (1988) (Lo-Lancet). Applicants respectfully rebut the rejection.

The Office Action describes Lo-Lancet as teaching the detection of donor-specific DNA in the plasma of kidney and liver transplant recipients, concluding that identification of Y chromosome-specific sequences in a female organ recipient using PCR with Y chromosome-specific primers is detecting an epigenetic difference.

As discussed above, detection of Y chromosomal sequences using Y chromosome-specific primers is detection of ***primary sequence*** characteristics, ***not epigenetic differences***. Moreover, as pointed out previously, there is no maternal homolog to the Y-chromosome and therefore its detection in a female host cannot be through comparison of epigenetic differences between the foreign and host DNA species. Accordingly, Lo-Lancet does not anticipate Applicants claimed invention and the rejection should be withdrawn.

Claims 1, 4, 6-8, and 25-27 are not anticipated by Mangioni *et al.*,

Claims 1, 4, 6-8, and 25-27 stand rejected under 35 U.S.C. §102(b) as allegedly

anticipated by Mangioni *et al.*, Bone Marrow Transplantation, 20, p. 969-973 (1997) (Mangioni). Applicants respectfully rebut the rejection.

Of relevance to the present invention, Mangioni discusses the use of a particular ***Y chromosome-specific primary nucleic acid sequence***, DYS14, in detecting residual male white blood cells in males after bone marrow transplant from a female donor. The test is to determine the level of host hemopoiesis after a myelo-ablative regimen. As noted in the Office Action, tests using the ***DYS14 primary nucleic acid sequence*** include PCR, Southern blot, and dot blot techniques.

Applicants respectfully remind the Examiner that it has long been understood by those of skill in the art that ***PCR, Southern blot, and dot blot techniques*** are founded on mutual recognition and hybridization between nucleic acids based on complimentary ***primary sequence recognition***. Unlike the methods of Applicants invention described above, the techniques discussed by Mangioni do not attempt to ***modify the nucleic acid*** to reflect an ***epigenetic difference*** (e.g., DNA methylation pattern). The Mangioni techniques are designed to recognize complimentary primary sequences ***without prior modification*** to reflect epigenetic differences. Therefore Mangioni does not teach methods for distinguishing epigenetic differences in nucleic acid species.

Furthermore, as DYS14 is a ***Y chromosome-specific marker***, DYS14 cannot be used as an epigenetic marker to ***differentiate*** between male and female DNA species, as there is ***no female homolog*** to the DYS14 marker. Applicants therefore respectfully request the current rejection be withdrawn.

Claims 1, 2, 4-5, 9-11, 13, 21-23, 27-29 are not anticipated by Kubota.

Claims 1, 2, 4-5, 9-11, 13, 21-23, 27-29 and 33 are rejected by the Examiner under 35 U.S.C. §102(b) as allegedly anticipated by Kubota *et al.*, Nature Genetics, 16, p. 16-17 (1997) (Kubota). Applicants respectfully rebut the rejection.

Kubota is described as "teaching" genomic imprinting, in the form of differential methylation between the maternal homolog and the paternal homolog, plays an important role in Prader-Willi syndrome (PWS) and Angelman syndrome (AS) (citing to p. 16, col. 1). Kubota is said to describe diagnosing the diseases by showing an affected ***individual*** lacks either a 100-bp PCR product (PWS) or a 174-bp PCR product (AS) present in a normal ***individual***. This description allegedly "teaches" a method of differentiating DNA species from different individuals in a blood sample by determining differing methylation patterns between the DNA species. Applicants respectfully disagree.

Kubota discusses a method of diagnosing diseases by taking advantage of methylation differences in the ***homologs*** of maternally and paternally inherited genes taken ***from the same individual***, not a method for determining differing methylation patterns between DNA species from ***different individuals***, as recited in Applicants claims.

Kubota identifies PWS and AS as genetic diseases, and describes a method of diagnosis that identifies aberrant structure or methylation of chromosome 15 (see p. 16, particularly fig 1). The Examiner acknowledges that the diagnosis identifies the 100-bp and 174-bp PCR products as originating from ***homologous chromosomes*** (maternal and paternal), and that only "normal" ***individuals*** display both the 174-bp and 100-bp PCR products. Diseased ***individuals*** lack one or both MSP products. (see Kubota, p16, cols 2 and 3).

Applicants respectfully point out that every ***individual*** possesses a pair of ***homologous chromosomes*** (one paternal, one maternal). Therefore, in referring to parental and maternal chromosomes, Kubota is discussing chromosome endogenous to

one individual, and not describing differentiating DNA species from ***different individuals***. ***Nowhere*** does Kubota discuss the use of differing methylation patterns as a means for differentiating DNA species from ***different individuals***, as recited in claim 1 of the instant application. Therefore, Kubota cannot anticipate Applicants claims and the rejection should be withdrawn.

Claim Rejections under 35 U.S.C. §103(a)

Combining Kubota and Herman does not render Claim 12 obvious.

The Examiner has rejected claim 12 under 35 U.S.C. § 103(a) as allegedly obvious over Kubota *et al* in view of Herman *et al.*, PNAS, 93, p. 9821-9826 (1996). Applicants respectfully traverse.

Establishing a prima facie case for obviousness under §103 requires the Examiner show, *inter alia*:

(1) The existence of some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988).

(2) A reasonable expectation of success in combining the references. This must be found in the prior art, and not in the applicants disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

(3) The prior art references teach or suggest all claim limitations of the rejected claim(s). *In re Royka*, 180 USPQ 580 (CCPA 1974); and MPEP §2143.03.

Kubota is described in the Office Action as teaching a method of differentiating DNA species from different individuals in a biological sample, namely blood, amniotic fluid or chorionic villus, by determining epigenetic differences.

Herman is described as teaching sequencing DNA after bisulfite treatment to "provide more direct analysis then [sic] MSP [methylation-specific PCR] for most

CpG sites..." The Office Action concludes that it would have been prima facie obvious to sequence CpG sites described in Kubota, as sequencing is "an equivalent means of determining methylation status," and provides "detailed information... of the methylation status of all CpG sites." Applicants respectfully disagree.

The combination fails to teach all elements of the present invention.

As discussed above in response to the anticipation rejection, Kubota describes a method of detecting aberrant methylation associated with PWS and AS in ***an individual***. Kubota does not suggest the use of epigenetic differences in distinguishing foreign DNA in a host.

Herman discuses the use of MSP as a tool in mapping DNA methylation patterns to better understand biological processes such as regulation of imprinted genes, X chromosome inactivation and tumor suppressor gene silencing in human cancer. Herman does not disclose differentiation of DNA species from different individuals in a biological sample.

As the combination of references suggested in the Office Action fails to provide all of the elements of Applicants claimed invention, a prima facie case of obviousness has not been set forth. Therefore Applicants respectfully request the rejection to claim 12 be withdrawn.

No motivation to make the suggested combination

Assuming *arguendo* that the suggested combination possessed all of the elements of Applicants invention, the obviousness rejection would still be improper as there is no motivation to make the suggested combination to arrive at Applicants invention.

Applicants respectfully point out Kubota's reasoning for choosing MSP as the analytical method of his assay was because:

> "[MSP] provides a reliable diagnostic method for all PWS and AS patients who show abnormal methylation at SNRPN. The ***major advantage*** of [MSP] is the ***rapidity*** of a PCR-based assay compared with a Southern Blot assay. This may have ***important implications*** for early diagnosis/management of hypotonic infants who may be suspected of having PWS, and for prenatal diagnosis." (Kubota col 2, p. 17.)

The Office Action proposes modifying the Kubota method by detecting methylation patterns by DNA sequencing, as allegedly taught by Herman, rather than with MSP as chosen by Kubota. Applicants respectfully suggest that the proposed combination, if workable, would frustrate rapid diagnosis of the condition ***sought by Kubota*** without providing any additional meaningful information.

The Office Action looks for support in Herman's statement that:

> "The only technique that can provide more direct analysis than MSP for most CpG sites within a defined region is genomic sequencing."

However, in the passage ***immediately*** after this statement Herman summarizes why ***DNA sequencing is inferior*** to MSP for mapping methylation patterns:

> "However, MSP can provide ***similar information*** and has the following advantages. First, MSP is much ***simpler*** and ***requires less time*** than genomic sequencing, with a typical PCR and gel analysis taking 4-6hr. In contrast, for genomic sequencing, amplification, cloning, and subsequent sequencing may take days. Second, MSP ***avoids*** the use of expensive sequencing reagents and the use of radioactivity. ... Third, the use of PCR as the step to distinguish methylated from unmethylated DNA in MSP allows for a significant ***increase in the sensitivity*** of methylation detection." (Herman at 9825-26.)

Herman then presents statistics illustrating the ***inferiority of DNA sequencing*** in determining methylation status of a CpG island, compared to MSP, concluding:

> "In summary, MSP is a ***simple, sensitive, and specific*** method for determining the methylation status of virtually any CpG-rich region." (Herman at 9826.)

In view of Herman's ***disparaging remarks*** regarding DNA sequencing in these types of assays, one of skill could not find motivation in the reference and in fact would have no reasonable expectation of success in making the suggested combination.

Moreover, as Herman describes DNA sequencing as an inferior analysis to MSP contradicting the Office Action conclusion that the two methods are "equivalent," and Kubota chose MSP over sequencing, Applicants respectfully suggest that the references are combined through impermissible hindsight, as the only motivation for combination resides in Applicants disclosure.

Combining Kubota and Nuovo does not render claims 30-32 obvious.

Claims 30-32 have been rejected by the Examiner under 35 U.S.C. § 103(a) as obvious over Kubota *et al* in view of Nuovo *et al.*, PNAS, 96, No. 22, p. 12754-12759 (1999).

The Office Action summarizes Kubota as above, adding that Kubota's method analyzes DNA from pregnant women to detect the presence of PWS and AS. Kubota does not describe differentiating between DNA species from different individuals in a biological sample.

Nuovo is described as teaching *in situ* detection of methylation using an *in situ* methylation-specific PCR (MSP-ISH). According to the Office Action, Nuovo applies MSP-ISH to tracing the evolution of cell populations harboring hypermethylation-associated inactivation, and identifying changes in specific cell types during embryonic development. Nuovo does not describe differentiating between DNA species from different individuals in a biological sample.

The Examiner concludes that it is obvious to modify and improve the method of Kubota to detect the presence of PWS and AS in pregnant women. The Examiner further believes that modifying Kubota to include MSP-ISH will provide

additional information regarding the precise timing of DNA methylation and change in specific cell types during embryonic development, as suggested by Nuovo.

Applicants respectfully disagree.

The combination fails to teach all elements of the present invention.

Neither Kubota nor Nuovo describe determining epigenetic differences between DNA species from different individuals, and therefore the combination cannot sustain a prima facie case of obviousness.

Claims 30-32 are dependent from claim 1 and incorporate all the elements and limitations of the parent claim(s). 35 U.S.C. §112 paragraph 4; and MPEP §608.01(n)(III). Claims 30-32 are therefore drawn to determining epigenetic differences between DNA species from different individuals in a biological sample.

As previously described, Kubota discusses a test for ***genetic aberrations*** associated with PWS and AS ***in individuals***. Kubota does not discuss differentiating between DNA species from different individuals in a biological sample.

Similarly, Nuovo generally describes a method of charting changes in DNA methylation patterns during tumor progression in an individual. Embryogenic study is mentioned speculatively as another possible use of the Nuovo method. Regardless Nuovo, like Kubota, provides no description of a method for determining epigenetic differences between DNA species from different individuals.

As Nuovo does not compensate for the deficiencies previously mentioned and found in Kubota, the proposed combination fails to teach each element as found in Applicants claims. As such, the Office Action fails to set forth a prima facie case of obviousness based on the cited references and the rejection should be withdrawn.

No motivation to make the suggested combination

Applicants respectfully suggest that there is no motivation in the references or the prior art for proposed combination.

As explained above, Kubota describes a simple, quick diagnostic method for identifying the genetic disorders PWS and AS. Kubota believes:

> "This may have important implications for early diagnosis/management of hypotonic infants who may be suspected of having PWS, and for prenatal diagnosis." (Kubota col 2, p. 17.)

Nuovo discusses methods of identifying and tracking hypermethylation of certain genes *in situ* using archived malignant cell preparations taken from an individual. These methods allow for the dissection of hypermethylation events in the progression of neoplastic tumors, and are postulated as being useful for the diagnosis of certain genetic disorders. (see page 12759 generally).

Nuovo discusses the MSP-ISH procedure on pp. 12754 -12755. The procedure includes making multiple sections from the tissue under study, performing MSP-ISH on each section, followed by microscopic analysis.

Again, assuming *arguendo* the combined references taught each element of Applicants claimed invention, Applicants respectfully suggest that proposed modification defeats the stated goal of Kubota, providing nothing to aid or confirm a simple, quick diagnosis. For example, the Nuovo method typically requires manual microscopic examination of samples that are time-consuming to conduct. Nuovo provides no suggestion of ways to avoid manual examination, and absent microscopic examination it is not clear how the combination would provide the advantage suggested in the Office Action as motivation, i.e., to "provide additional information regarding the precise timing of DNA methylation and change in specific cell types during embryonic development." Therefore, as the Office Action fails to indicate where motivation for the combination lies in the prior art or the references sought to be combined, a prima facie case of obviousness has not been established, and the rejection should be withdrawn.

Obviousness-type Double Patenting

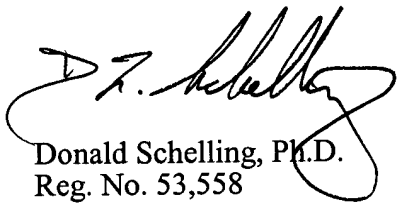
Claims 1, 3-5, 8, 14-20, 24, 27 and 33 unpatentable over claims 1-7, 12-25 of U.S. Pat. No.: 6,258,540. This is essentially the same rejection as the one made by the Examiner under 35 U.S.C. § 102 rejection based on this patent above. Applicants therefore respond in the same manner here, as above and request the rejection be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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